

THE INFLUENCE OF STRESS ON THE APPETITIVE
NATURE OF DRUG CUES, SKIN CONDUCTANCE, AND
HEART RATE IN A NICOTINE
DEPENDENT SAMPLE.

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CHAPTER I

INTRODUCTION

Introduction

Despite recent declines in rates of smoking in the U.S., approximately 20.6% of the population are still current smokers, resulting in 443,000 smoking related deaths per year (Dube SR, 2010). Additionally, reports have indicated a five year stall in the decline in smoking rates observed in past years (Dube SR, 2010). Recent advances in pharmacological, educational, and behavioral interventions have been helpful in reducing smoking rates, however objective measures of these interventions are still lacking (Volkow, 2004). The current measure of success or failure for smoking interventions typically includes self report and behavioral measures indicating when an individual has failed in their quit attempt (Volkow, 2004). Psychophysiological measures may provide important information for an individual's success or lack thereof, in treatment. Specifically, psychophysiological measures may provide a more objective alternative to self report data, and may provide useful information about the efficacy of an intervention before the individual relapses.

The affective modulation of the startle response (AMSR) has been proposed as an objective measure of motivational valence for smoking cues (Geier, Mucha, & Pauli, 2000). This approach categorizes cues as appetitive (pleasant), neutral, or aversive (unpleasant). When the startle response is elicited while an individual is viewing an experimental cue that is inherently pleasant to them their reflexive response is diminished. Conversely, when the startle response is elicited while an individual is viewing an image

that is inherently unpleasant to them the reflexive response is increased. The startle response has been used to examine the effects of nicotine on the physiological responsiveness of the muscular system for some time (Gilbert & Hagen, 1980). However, the startle response has only recently been used to examine the emotionally based aversive or appetitive nature of smoking cues. Geier and colleagues (2000) found that smoking cues attenuated the startle response in dependent smokers as compared to neutral and unpleasant pictures, indicating that the cues are appetitive in nature. The findings of the Geier 2000 study were replicated by Dempsey and colleagues (2007), supporting the previous findings that smoking images attenuate the startle response. Thus, the extant data indicate that smoking cues are appetitive in nature and promotes this paradigm as a potentially valuable tool for objectively elucidating mechanisms involved in nicotine addiction.

Additionally, other psychophysiological measures have been used to examine an individual's affective response to pleasant, unpleasant, and neutral stimuli. Examining other variables may help to solidify or refute findings observed in startle response data. Previous studies have found that electrodermal responses are sensitive to the rated arousal of pictures, and provide a measure of stimulus activation or intensity level (Lang, Greenwald, Bradley, & Hamm, 1993). Large skin conductance responses occur to both positive and negative images. The magnitude of the response depends on how arousing the stimulus is to the participant, and is independent of the positive or negative nature of the stimulus (Lang, et al., 1993). Heart rate has also been shown to change based on the affective nature of a stimulus. A deceleration in heart rate during the first two seconds of presentation of a novel stimulus has been reported (M. M. Bradley, Lang, & Cuthbert, 1993). This heart rate deceleration is the result of an individual's orienting response, and

demonstrates that the individual is attending to the presented stimulus. Heart rate also changes reliably based on the rated pleasure of specific stimuli after the initial orienting response, with increases in rated pleasure resulting in cardiac acceleration, and increases in rated unpleasantness resulting in cardiac deceleration (M. M. Bradley, et al., 1993).

The current study proposed to test the viability of the affective modulation of the startle response paradigm as a tool for examining the influence of stress on the established appetitive nature of nicotine cues. Additionally, the ability of smoking images and stress to influence measures of skin conductance and heart rate were also examined. The paradigm's ability to examine the influence of *stress* on the appetitive nature of nicotine cues was selected due to the well established relationship between stress and nicotine maintenance and relapse (Buczek, Le, Wang, Stewart, & Shaham, 1999; S. Cohen & Lichtenstein, 1990; Morse, 1989; O. F. Pomerleau & Pomerleau, 1991).

Stress can be defined as any imbalance of homeostasis and includes activation of the parasympathetic and sympathetic nervous systems (Tsigos & Chrousos, 2002) as well as the hypothalamic pituitary adrenal (HPA) axis (G. F. Koob, 2009). The parasympathetic nervous system generally regulates organs and glands while the sympathetic nervous system increases heart rate, respiration, and skin conductance amongst other processes (Dawson, 1990). The HPA-axis controls reactions to stress and includes communication between the hypothalamus, pituitary gland, and the adrenal glands. As with most addictive substances, acute withdrawal from nicotine initially increases activity in the HPA-axis (Richter & Weiss, 1999). This increase in HPA-axis activity is thought to influence motivation to reinstate the substance via negative reinforcement as administration of nicotine would end the unpleasant feelings associated with the stress involved in

withdrawal (G. F. Koob, 2009). Over time, smokers learn that nicotine diminishes the negative feelings that are experienced during withdrawal. Stress from the outside environment results in the same HPA-axis activation that withdrawal from nicotine does. Therefore, it is not surprising that smokers may be cued to administer nicotine in order to reduce the feelings of stress caused by the outside environment based on previous learning that nicotine reduces these negative feelings when the individual is experiencing acute withdrawal (Grunberg, 2007). Following this argument, it is clear why increased stress has been shown to be positively correlated with self reported urges to smoke (Swan, Ward, Jack, & Javitz, 1993; Tiffany, 1990). Laboratory experiments have shown that corticotrophin releasing factor (a key neuropeptide in the HPA stress response) antagonists block the negative symptoms that normally occur during nicotine withdrawal, adding support to the argument that this stress system is largely responsible for the negative reinforcement which maintains nicotine dependence (Tucci, Genn, Marco, & File, 2003). Furthermore, studies have demonstrated that stress causes short term changes of circuitry in the mesolimbic reward pathway (Piazza & Le Moal, 1998). Thus, stress may prime the brain for reward, which would increase the reinforcing effects of nicotine in dependent individuals (Piazza & Le Moal, 1998).

Despite a plethora of studies indicating a relationship between stress and nicotine addiction, the behavioral mechanisms by which stress exposure increases drug use, including nicotine use, remain undetermined (Sinha, 2001). The current study proposes to elucidate one possible mechanism that may increase nicotine use in response to stress. Specifically, it is hypothesized that the learned effect of nicotine's ability to decrease negative symptoms associated with HPA-axis activity, as well as the increased sensitivity

to nicotine during times of HPA-axis activity maintains smoking behavior by increasing the pleasurable affective valence of smoking cues during periods of HPA-axis activity.

The first hypothesis was tested by examining the affective modulation of the startle reflex to nicotine cues in nicotine dependent individuals exposed to a stress task which has reliably been shown to increase HPA-axis activity (Buchanan & Tranel, 2008; Kirschbaum, Pirke, & Hellhammer, 1993). Specifically, the startle magnitude of nicotine dependent individuals was measured while viewing positive, neutral, negative, and smoking cues directly after completing a stress task or conversely a physiologically neutral control condition. It was hypothesized that if stress modulates the appetitive nature of nicotine cues, the individuals exposed to the stress task should evince significantly diminished startle reflexes to nicotine cues as compared to those in the control condition.

Given the findings of previous research indicating the ability of affective stimuli as well as stress to affect other psychophysiological measures, several additional hypotheses were developed. It was hypothesized that participants in the stress condition would evince a decrease in tonic skin conductance levels over time following the experimental manipulation compared to those in the control condition, as galvanic skin conductance is controlled by the sympathetic nervous system and is associated with the physiological arousal observed in stressful tasks (Duffy, 1957). This hypothesis was formed to serve as a manipulation check of physiological activation in the stress condition. Significant increases in phasic skin conductance responses for smoking images in the stress condition as compared to the control condition were expected as galvanic skin responses are significantly potentiated to novel pleasant and unpleasant images as compared to novel neutral images (M. M. Bradley, 2009). Finally it was hypothesized that smoking images

would significantly increase heart rate for the stress group in comparison to the control group, as increased heart rate has been observed in response to pleasant images (Bradley, 2009).

CHAPTER II

REVIEW OF LITERATURE

Overview of Addiction

Substance Abuse

The severity of substance use is typically determined by frequency of use as well as the degree to which the substance use interferes with or impairs the life of an individual. This range is typically defined by three qualitative degrees; use, abuse, and dependence.

Substance use is the administration of any potentially harmful or addictive substance. Commonly used substances of abuse include: alcohol; amphetamine or similarly acting sympathomimetics; cannabis, cocaine, hallucinogens, inhalants, nicotine, opioids, phencyclidine, sedatives, hypnotics, and anxiolytics.

Substance abuse is currently defined in the Diagnostic and Statistical Manual of Mental Disorders as: “A maladaptive pattern of substance use manifested by recurrent and significant adverse consequences related to the repeated use of substances” (APA, 2000). In order for Abuse criteria to be met, the substance-related problem must have occurred repeatedly during the same 12 month period or been persistent (APA, 2000). There may be repeated failure to fulfill major role obligations, repeated use in situations during which it is physically hazardous, multiple legal problems, and recurrent social and interpersonal problems (APA, 2000). According to DSM-IV-TR the maladaptive pattern of substance use leading to clinically significant impairment or distress is manifested by one (or more) of the following, occurring

within a 12-month period: (1) Recurrent substance use resulting in a failure to fulfill major role (2) Recurrent substance use in situations in which it is physically hazardous (3) recurrent substance-related legal problems (4) Continued substance use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of the substance. Although other definitions exist, e.g. World Health Organization, most are similar to the one given in DSM-IV-TR.

Substance Dependence

The terms addiction and substance dependence are used interchangeably in the literature unless otherwise specified. There are two current operational definitions endorsed by major health organizations that are relevant to this review. The American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV) and the World Health Organization's International Statistical Classification of Diseases and Related Health Problems Tenth Edition (ICD-10) contain definitions of substance dependence / addiction that are widely used by clinicians (WHO, 2004).

Substance dependence is currently defined in the Diagnostic and Statistical Manual of Mental Disorders as: "A cluster of cognitive, behavioral, and physiological symptoms indicating that the individual continues use of the substance despite significant substance related problems" (DSM-IV-TR). There is a pattern of repeated self-administration that can result in tolerance, withdrawal, and compulsive drug taking behavior (DSM-IV-TR). The DSM-IV-TR lists the following: "the maladaptive pattern of substance use leading to clinically significant impairment or distress, is manifested by three (or more) of the following, occurring any time in the same 12-month period: (1) Tolerance (2) Withdrawal

(3) The substance is often taken in larger amounts or over a longer period than intended (4) there is a persistent desire or unsuccessful efforts to cut down or control substance use (5) a great deal of time is spent in activities necessary to obtain the substance, use the substance, or recover from its effects (6) important social, occupational, or recreational activities are given up or reduced because of substance use (7) the substance use is continued despite knowledge of having a persistent physical or psychological (DSM-IV-TR).

The world health organization's International Statistical Classification of Diseases and Related Health Problems defines substance dependence: "as a cluster of physiological, behavioral, and cognitive phenomena in which the use of a substance or a class of substances takes on a much higher priority for a given individual than other behaviors that once had greater value" (WHO, 2004). The ICD-10 notes that: "a central descriptive characteristic of the dependence syndrome is the desire (often strong, sometimes overpowering) to take psychoactive drugs (which may or may not have been medically prescribed), alcohol, or tobacco (ICD-10). The ICD- 10 notes that: there may be evidence that return to substance use after a period of abstinence leads to a more rapid reappearance of other features of the syndrome than occurs with nondependent individuals. A definite diagnosis of dependence should usually be made only if three or more of the following have been present together at some time during the previous year: (1) A strong desire or sense of compulsion to take the substance; (2) Difficulties in controlling substance-taking behavior in terms of its onset, termination, or levels of use; (3) A physiological withdrawal state when substance use has ceased or have been reduced, as evidenced by: (a) the characteristic withdrawal syndrome for the substance; or use of the same (or closely

related) substance with the intention of relieving or avoiding withdrawal symptoms; (b) Evidence of tolerance, such that increased doses of the psychoactive substance are required in order to achieve effects originally produced by lower doses; (c) Progressive neglect of alternative pleasures or interests because of psychoactive substance use, increased amount of time necessary to obtain or take the substance or to recover from its effects; (d) Persisting with substance use despite clear evidence of overtly harmful consequences, such as harm to the liver through excessive drinking, depressive mood states consequent to periods of heavy substance use, or drug-related impairment of cognitive functioning; efforts should be made to determine that the user was actually, or could be expected to be, aware of the nature and extent of the harm (ICD 10).

While these definitions are useful in diagnosing individuals with substance dependence and recognizing symptoms of the disorder, they do not give us much insight into the underlying causes of the behavior. In order to understand the processes involved in addiction it is helpful to set some conceptual framework.

Concepts in Substance abuse and Dependence

While there are a multitude of theories and conceptualizations of the specific mechanisms involved in substance dependence, the majority focus on many of the same basic elements. Positive reinforcement in substance use is established when administration of the substance causes the release of the rewarding neurotransmitter dopamine (Kalivas & Volkow, 2005; Markou, 2008; See, Fuchs, Ledford, & McLaughlin, 2003). Continued use of the substance often results in physiological tolerance and withdrawal symptoms. Tolerance is the need to increase the amount of the substance to achieve the same effect, or

the experience of a diminished effect if continuing to administer the same amount of the substance over time. Withdrawal syndromes differ with specific substances but are the result of cessation of administration of the substance. Symptoms of withdrawal range from mild headache to severe health complications including death. Negative Reinforcement occurs when a substance is administered in order to avoid or reduce the unpleasant symptoms of physiological withdrawal. The repeated administration of an addictive substance can result in dysregulation of the brain's reward pathways leading to impulsive and compulsive drug taking behavior (G. Koob & Kreek, 2007). Impulsive behavior has been defined as rapid, unplanned reactions to internal and external stimuli without regard for the negative consequences of these reactions to the individual or others (G. F. Koob, 2009). Compulsive behavior has been defined as perseveration in responding in the face of adverse consequences or perseveration in the face of incorrect responses in choice situations (G. F. Koob, 2009). All of the concepts previously mentioned are posited to occur through a maladaptive form of the brain plasticity that normally allows us to benefit and learn from our experiences in useful ways (Kalivas & Volkow, 2005). All of these processes and elements occur directly in or are mediated by a part of the brain called the mesolimbic pathway (Adinoff, 2004).

Disputed forms of addiction

Some behaviors that share many of the unhealthy and repetitive patterns commonly seen in substance abuse have been proposed as new forms of addiction. These behaviors often appear to successfully fit the diagnostic criteria for substance abuse or dependence as well as the theoretical models. Pathological gambling and Sexual Addiction are currently disputed in the field as valid forms of addiction.

Pathological gambling is found in the DSM-IV-TR under Impulse Control Disorders Not Elsewhere Classified. Pathological gambling is a persistent and recurrent maladaptive gambling behavior that disrupts personal, family, or vocational pursuits (DSM-IV-TR). The ICD-10 classifies pathological gambling under Habit and Impulse Disorders and defines it as frequent, repeated episodes of gambling that dominate the patient's life to the detriment of social, occupational, material, and family values and commitments. Pathological gambling often resembles the same elements of impulsivity and compulsivity seen in substance dependence (Potenza, 2008). As in substance dependence, this impulsive and compulsive behavior is hypothesized to be the result of a dysregulation of the brain's mesolimbic reward pathway. The classification of this behavior as an addiction is still disputed amongst experts in the field because of its failure to match many facets of the traditional addiction framework (e.g. tolerance and withdrawal).

The DSM-IV-TR does not currently classify sexual addiction as a mental disorder. However, sexual addiction is described in an example under the classification of Sexual Disorders Not Otherwise Specified as "distress about a pattern of repeated sexual relationships involving a succession of lovers who are experienced by the individual only as things to be used." The ICD-10 includes the classification Excessive Sexual Drive under Sexual dysfunction not caused by organic disorder or disease. It is subdivided into satyriasis in men and nymphomania in women. The familiar patterns of impulsivity and compulsivity exhibited in Pathological Gambling as well as Substance Dependence are present in individuals said to have a sexual addiction (Goodman, 1993). An imbalance in the mesolimbic reward system, associated with chemical abuse and dependence, has also been implicated in this phenomenon (Crocq, 2007). However, as is the case with

pathological gambling, this maladaptive sexual behavior is still disputed as an addiction because of its failure to meet many of the core principles of substance addiction.

Specific Drugs of Abuse

Opioids

While some consider opioids to be a depressant, other researchers include this substance as its own category, analgesics. Opium is a derivative of the poppy seed and has been used for thousands of years for medicine and recreation. Morphine was isolated from opium as its active ingredient in the early 1800's and has been used throughout the world for treating severe pain (Julien, 2005). Opioids mimic the actions of endogenous endorphins by binding to their sites which are distributed throughout the central nervous system (Julien, 2005). There are three types of these receptor sites called mu, kappa, and delta. The mu receptors seem to have the highest reinforcement mechanism of the three and therefore are often implicated as playing the most significant role in the addictive properties of the opioids (Kieffer, 1999). Heroin is synthesized from morphine and is the most widely abused illicit form of opioids (Julien, 2005). Many prescription drugs prescribed for severe pain contain opioids; including well known brand names such as Vicodin, Percocet, and Oxycontin. These opioid containing prescription medications are abused at high rates and often result in dependence. In 2008, 453,000 Americans age 12 and older had abused heroin at least once in the year prior to being surveyed (SAMSHA, 2008).

Depressants: Alcohol

Alcohol has been used for medicine and recreation since before recorded history. The exact mechanism of alcohol is still being researched today; however basic research has shown that many receptors are involved. Alcohol acts on at least 6 known receptor sites including; glutamate, GABA, opioid, serotonin and cannabinoid receptors. Ethanol inhibits the NMDA-subtype of glutamate receptors, which play a large role in severe withdrawal symptoms upon alcohol cessation (Tsai & Coyle, 1998). Ethanol activates the GABA-mediated increase in chloride ion flows, resulting in neuronal inhibition (Feldman, 1997). It has been posited that ethanol induces opioid release which in turn triggers dopamine release in the brain reward system (Wand, Mangold, El Deiry, McCaul, & Hoover, 1998). It has been shown in a number of studies that chronic use of ethanol results in disturbances in serotonergic activity and this is thought to play a role in the pathogenesis of various presentations of alcohol dependence (Julien, 2005). Finally, the chronic ingestion of ethanol has been shown to stimulate the endogenous neurotransmitter for cannabinoids called anandamide (Julien, 2005). Anandamide depletion after cessation of ethanol use is thought to produce craving symptoms (Hungund & Basavarajappa, 2000). In 2008, 51.6% of Americans age 12 and older had used alcohol at least once in the 30 days prior to being surveyed; 23.3% had binged (5+ drinks within 2 hours); and 23.3% drank heavily (5+ drinks on 5+ occasions). In the 12-17 age range, 14.6% had consumed at least one drink in the 30 days prior to being surveyed; 8.8% had binged; and 2.0% drank heavily (SAMSHA, 2008).

Depressants: Benzodiazepines

Benzodiazepines were discovered in 1955 and introduced to the general public in the 1960's. Well known for their anxiolytic properties, common benzodiazepines include

diazepam (Valium), alprazolam (Xanax), clonazepam (Klonopin), lorazepam (Ativan), and triazolam (Halcion). They are all agonists of the GABA-benzodiazepine-chloride receptor complex which facilitates the binding of GABA (Haefely, 1989). This GABA binding facilitates the influx of chloride ions which causes hyperpolarization of the postsynaptic neuron, depressing its excitability (Julien, 2005). Benzodiazepines produce their anxiolytic properties by acting on the limbic centers of the brain (Adinoff, 2004). While it is difficult to determine the prevalence of abuse of this drug, it is noted as one of the most commonly abused prescription medicines (Ashton, 2005). In 2008, 15.2 million Americans age 12 and older had taken a prescription pain reliever, tranquilizer, stimulant, or sedative for nonmedical purposes at least once in the year prior to being surveyed (SAMSHA, 2008).

Stimulants: Cocaine

Cocaine is obtained from the leaves of the coca plant. Cocaine's physiological and psychological effects come from its ability to block the reuptake of dopamine, norepinephrine, and serotonin into presynaptic terminals (Wieczorek & Kruk, 1994). As a result of this inhibition cocaine potentiates the neurotransmission of all three neurotransmitters (Ross & Renyi, 1969). The major behavioral effect of cocaine is that of a psycho stimulant (Johanson & Fischman, 1989). This behavior is produced primarily by the blockage of dopamine reuptake but it has been shown that serotonin plays a role as well (Rocha, et al., 1998). Increased dopamine levels in the nucleus accumbens and other components of the dopaminergic reward system seem to be responsible for the euphoric and addictive effects of the drug (Wise, 1998). In 2008, 5.3 million Americans age 12 and older had abused cocaine in any form and 1.1 million had abused cocaine in smoked form at least once in the year prior to being surveyed (SAMSHA, 2008).

Stimulants: Amphetamines

Amphetamine was first synthesized in 1887 and was readily available over the counter in the United States until the 1950's. Today amphetamines are only available by prescription, and are used regularly to treat conditions such as Attention Deficit Hyperactivity Disorder. The therapeutic use of amphetamines often turns into abuse and dependence (Dopheide & Pliszka, 2009). The related compound methamphetamine was first synthesized in Japan in 1918. Methamphetamine is now produced through a number of methods by altering their precursor drugs ephedrine or pseudoephedrine. Amphetamine increases the amount of dopamine in the synaptic cleft in a number of ways. It induces the release of dopamine by binding to the pre-synaptic membrane of dopaminergic neurons, it interacts with synaptic vesicles releasing free dopamine into the nerve terminal, it prevents the degradation of dopamine by binding to monoamine oxidase in dopaminergic neurons, and it binds to the dopamine re-uptake transporter causing it to act in reverse and transport free dopamine out of the nerve terminal (King, 1997). In the United States over 850,000 people age 12 or older have abused methamphetamine in the previous year (SAMSHA, 2008).

Stimulants: Nicotine

Nicotinic receptors modulate the effects of a wide range of neurotransmitter pathways, including the cholinergic system itself, as well as the dopamine, glutamate, GABA, 5-HT, norepinephrine, opioid, and histaminergic systems (Kumari & Postma, 2005). Nicotine acetylcholine receptors are the major sites that nicotine uses to exert its behavioral effects (Kumari & Postma, 2005). Animal studies have shown that several brain areas are rich in nicotine acetylcholine receptors including the hypothalamus,

hippocampus, thalamus, midbrain, brainstem and areas of cerebral cortex (Clarke, 1993). Like many of the substances previously mentioned, nicotine's reinforcing properties are believed to come from its interaction with the mesolimbic pathways, specifically through an increase of dopamine in the nucleus accumbens (Balfour, 2009). Approximately 22% of the U.S. population are current smokers (SAMSHA, 2008).

Nicotine Use Disorders

Nicotine Abuse: Chippers

Although most tobacco users eventually become dependent on the drug, a subset of individuals who use tobacco on a regular basis show few if any objective signs of nicotine dependence (Shiffman, 1989). These individuals are referred to as chippers and are generally defined as those who smoke only a few cigarettes per day but smoke more than half of the days of the week. Chippers exhibit many of the same physiological responses to smoking tobacco as do heavy smokers. However, when in a tobacco deprived state, chippers, unlike heavy smokers, do not typically show signs of nicotine withdrawal (Shiffman, Paty, Gnys, Kassel, & Elash, 1995).

Nicotine Abuse: Self-Medication

The self-medication hypothesis of addictive disorders was originally derived by Edward Khantzian and David Duncan in 1974 through their work with cocaine and opiate abusing patients. According to the theory, individuals may use substances for therapeutic purposes, (i.e. to alleviate psychological or physical pain), without professional advice or knowledge. Individuals discover that the specific actions or effects of each class of drugs relieve or change a range of painful affective states (Khantzian, 1985). Khantzian (1985)

noted that self medication behaviors most often result from an individual's difficulty in regulating emotional states related to self esteem, relationships, and self care. Studies examining self medication theory have found varying levels of support depending on the type of disorder or condition that the individual is suffering from.

Nicotine Dependence

The core principles of general substance dependence defined previously apply to nicotine dependence as well. The DSM-IV-TR refers the reader to the criteria for general substance dependence and states: "some of the generic dependence criteria do not appear to apply to nicotine, whereas others require further explanation". The DSM-IV-TR provides examples of adaptations of the general dependence criteria made to more accurately characterize nicotine dependence. The DSM-IV-TR also notes chain smoking as an indicator of excessive time spent smoking and lists continued use of tobacco despite knowledge of medical problems related to the use as a particularly important health problem (DSM-IV-TR).

The World Health Organization's ICD 10 refers to Nicotine Dependence as Tobacco Dependence Syndrome and describes it as: "a cluster of physiological, behavioral, and cognitive phenomena in which the use of tobacco takes on a much higher priority for a given individual than other behaviors that once had greater value". The manual describes a strong "desire" to use tobacco as a central characteristic of the syndrome (ICD-10). The ICD-10 also notes that the reinstatement of the drug after a period of abstinence often results in quicker onset of dependence symptoms (ICD-10).

Nicotine Dependence Risk Factors

Risk Factors – Genetic Influence

It has been reported that up to 56% of the variance in nicotine dependence is associated with heritability (Li, Cheng, Ma, & Swan, 2003). Some specific genes have been identified as mediators of smoking behavior. The cytochrome P450 CYP2A6 gene which metabolizes nicotine to cotinine has been implicated (Ray, Schnoll, & Lerman, 2009). A review by Ray and colleagues (2009) found that individuals with the CYP2A6 wild type genotype, smoke more cigarettes per day, have greater Fagerstrom Tobacco Test for Nicotine Dependence scores, and are less likely to quit smoking (Gu, Hinks, Morton, & Day, 2000). This wild type genotype results in faster metabolism of nicotine, which likely leads to these effects on behavior. The Nicotine Acetylcholine Receptor (nAChR) subunit genes have also been posited as important genes influencing susceptibility to nicotine dependence. Associations have been reported between nicotine dependence and the nAChR subunit genes CHRNA4, CHRNA5, and CHRNA3 (Berrettini, et al., 2008; Saccone, et al., 2007). In addition to genes directly associated with nicotine dependence, genes that mediate activity in the dopamine reward system may play a role in nicotine dependence as well.

Risk Factors - Hormonal Influence

Many studies have evaluated the effect of hormones on nicotine dependence. While the animal literature provides strong evidence for the role of hormones in addictive behaviors, the clinical literature is mixed (S. S. Allen, Allen, Lunos, & Hatsukami, 2009). Animal studies have found that estrogen increases the self administration of drugs while progesterone decreases it (Anker, Larson, Gliddon, & Carroll, 2007; Lynch, Roth,

Mickelberg, & Carroll, 2001). Results are inconsistent regarding associations between attempts at smoking cessation and estrogen and progesterone levels as indicated by menstrual cycle phase. Some studies have identified the follicular phase (high estrogen, low progesterone) to be associated with poorer smoking cessation outcomes (C. D. Allen & Cyster, 2008), whereas others have found that the luteal phase (low estrogen, high progesterone) is associated with poor outcomes (Carpenter, Saladin, Leinbach, Larowe, & Upadhyaya, 2008; Franklin, et al., 2008).

Risk Factors - Psychopathology

A positive association between depression and cigarette smoking has been well established (Lerman, et al., 1998). Individuals who have a history of major depressive disorder are significantly more likely to have a comorbid disorder of nicotine dependence compared to non-depressed individuals (Breslau, Peterson, Schultz, Chilcoat, & Andreski, 1998). Smokers are more likely than nonsmokers to report depressive symptoms (Perez-Stable, Marin, Marin, & Katz, 1990). The presence of depressive symptoms increases the likelihood of relapse once a quit attempt has been made (Glassman, et al., 1990; Hall, Munoz, Reus, & Sees, 1993). The self medication hypothesis (detailed in section “Nicotine Use: Self – Medication”) has been posited to explain this association between nicotine dependence and depression (Khantzian, 1985). Pomerleau and colleagues (1994) proposed that the mood altering effects of nicotine are especially salient in depressed individuals.

A number of researchers have found evidence linking cigarette smoking and anxiety (Morissette, Tull, Gulliver, Kamholz, & Zimering, 2007; Zvolensky, et al., 2006). Lerman and colleagues (1998) note that despite evidence that smoking elevates peripheral autonomic nervous system activity, cigarette smokers commonly report smoking to reduce

negative effect, or "calm down." The apparent reduction in anxiety after smoking is believed to be due to mood normalization after extinguishing short term withdrawal symptoms (Parrott, 1999). Many studies have noted the increased likelihood of individuals with elevated anxiety to use anxiolytic substances; however studies examining the anxiolytic properties of nicotine are inconsistent.

An extremely high rate of nicotine use is observed in Schizophrenic patients as compared to healthy populations. Nicotine does not substantially alleviate the principle symptoms of schizophrenia; therefore it is unclear why such a high rate of this population smokes. One theory of why nicotine use is so common amongst schizophrenics has to do with a deficit they have in which they experience trouble maintaining acute attention and focusing properly on sensory information. This is called an impairment in sensory gating, and schizophrenic patients describe it as an uncomfortable feeling that "information is coming in too quickly" (Light & Braff, 1999). Adler and colleagues (1993) showed that cigarette smoking can temporarily normalize the impairment of this sensory gating problem, which would explain why it is so prevalent in the schizophrenic population.

Relapse in Nicotine Dependence

Zhou and colleagues (2009) describe smoking cessation as a dynamic process that often involves a sequence of unsuccessful attempts to quit before long-term abstinence is achieved. Approximately 75% to 80% of smokers who attempt to quit relapse before achieving 6 months of abstinence, additionally relapse may occur years after the initial quit date (Zhou, et al., 2009). Zhou and colleagues (2009) found that higher motivation and intent to quit predicts making a quit attempt but is not associated with subsequent relapse.

Smokers who have recently made a failed attempt to quit are more likely to try again but also more likely to relapse than those who have not tried recently (Zhou, et al., 2009).

Many independent factors have been implicated in smoking relapse. Smoker's subjective ratings of their cravings for cigarettes immediately following smoking cessation have been shown to be positively correlated with rates of smoking relapse (Killen & Fortmann, 1997). A study examining the role of impulsivity in smoking relapse found that individuals with higher levels of trait impulsivity evinced a shorter time to relapse than their low impulsivity counterparts (Doran, Spring, McChargue, Pergadia, & Richmond, 2004). A study examining negative affective states preceding relapse found that 13.1% of individuals relapsed after feeling depressed, 33.3% of individuals relapsed after feeling anxious or experiencing feelings of tension, and 15.5% of individuals relapsed after feeling anger or irritation (Brandon, Tiffany, Obremski, & Baker, 1990). Stress has also been noted as one of the most common emotional states preceding relapse and will be discussed in detail in the section "Stress in Nicotine Relapse".

Measuring Nicotine Dependence

Biological Markers vs. Subjective Reports

While the standard method for assessing nicotine dependence is through self reported subjective measures, the identification of a biomarker of tobacco, i.e. a metabolite found in saliva, can aid in reducing the inherent problems of self reports. However, biomarkers do have some physical limitations which can prove to be problematic in common research paradigms. Often times they are expired within short time periods after exposure to the substance being measured. For example, cocaine's main metabolite

benzoylcoine is typically eliminated from the body within 72 hours of drug administration. Therefore, urine samples must be collected frequently in order to accurately monitor drug use. Additionally, there may be factors influencing the increase or decrease in concentration of the chemical marker other than the variable that is being measured. For example, cortisol is a common biomarker used to measure stress levels; however it can be influenced by factors other than stress such as menstrual cycle and nicotine use. Common biological markers for nicotine include cotinine and expired carbon monoxide.

Measuring: Biological Markers

The most widely used biological marker of nicotine use is called cotinine (Benowitz, 2008). Cotinine is the first-stage metabolite of nicotine and can be found in the blood, urine, saliva, hair, or nails. The recommended cut point used to distinguish smokers from non-smokers in the general US population is 3ng/ml (Benowitz, 2008). Cotinine levels are fairly stable throughout the day in smokers, in contrast to expired nicotine levels which fluctuate largely depending on urine Ph (Benowitz, 2008). As a result cotinine is considered to be the best biological marker for determining nicotine use.

Smoked tobacco causes a buildup of the poisonous chemical carbon monoxide in the lungs. Measuring the amount of carbon monoxide in the air expired by an individual gives a short term estimate of exposure to smoking (Leone, 2005). This measure is often used as a valuable motivational tool in smoking cessation programs. It can also be used to corroborate self report information; however it cannot be taken on its own as a valid indicator of smoking cessation due to its temporal limitations.

Self Report Measures of Nicotine Dependence

The benefit of subjective measures of self report is their freedom from the physical limitations of biological markers of nicotine dependence discussed previously. The drawbacks of self report measures include poor sensitivity and specificity possibly due to an individual's failure to disclose nicotine use as well as poor history recollection of use (Schuler, Lechner, Carter, & Malcolm, 2009).

The Fagerstrom Test for Nicotine Dependence (FTND), an adaptation of the Fagerstrom Tolerance Questionnaire (FTQ), provides a short convenient self-report measure of dependency on nicotine. Pomerleau and colleagues assessed the test-retest stability of both the FTND and FTQ in 2 samples: 237 smokers in a laboratory setting and 36 smokers hospitalized for depression. The reliability of both scales was high in both groups (C. S. Pomerleau, Carton, Lutzke, Flessland, & Pomerleau, 1994). The validity of the scales as assessed by multiple methods including cotinine levels and self reported years smoked was supported (C. S. Pomerleau, et al., 1994). There are two bases of scoring for The Fagerstrom Test for Nicotine Dependence; one for adults and one for Adolescents. The Adolescent form assigns higher points to indicators of dependence. Scores of 1-2 indicate low dependence, scores of 3 to 4 indicate low to moderate dependence, scores of 5 to 7 indicate moderate to high dependence and scores of 8 or more indicate high dependence (Heatherton, 1991).

The Time Line Follow Back is a method for assessing recent drinking and substance use behavior. The TLFB can be administered by an interviewer, self-administered or administered by computer. It involves asking clients to retrospectively

estimate their daily nicotine consumption over a time period prior to the interview. This self report measure can offer invaluable data to the extent that participants are willing to disclose their smoking behavior, and they are accurate historians of their smoking behavior.

Stress

Defining Psychosocial Stress

There are many definitions of the construct of stress provided in the literature. Psychologists describe stress as the difficulty involved in adapting to the demands of one's environment (Selye, 1975). Stress is also described as a response to any discrepancy between what an organism is expecting and what actually exists (Goeders, 2003). Others define stress as a lack of necessary resources to complete a task or goal (Miller & O'Callaghan, 2002). An individual's response to stress is as varied as its definitions. While most researchers would agree that stress can be qualitatively construed as being part of a core fundamental affective response, numerous physiological reactions in response to stress can be quantified in the individual. Most reactions to stress involve the sympathetic nervous system and the hypothalamo-pituitary-adrenal (HPA) axis. This physiological stress response is necessary for allowing individuals to respond appropriately to environmental demands. Activation of the sympathetic nervous system results in a number of physiological changes including: increased heart rate, increased blood pressure, dilation of pupils, and increased respiration. This system is preparing the body to react once the choice is made to fight or flight in response to a stressor. There are a number of systems involved in the reaction to and regulation of stress including the hypothalamus, pituitary

gland, and the adrenal gland, which are commonly referred to as the HPA axis. The HPA axis is stimulated by the corticotrophin releasing hormone (CRH) in response to stress (Goeders, 2003). CRH is released into the adeno physeal portal circulation which results in synthesis of adrenocorticotropin hormone, (ACTH) which in turn stimulates the biosynthesis and secretion of adrenocorticosteroids (cortisol in humans) (Goeders, 2003). This communication system directs action in order to keep our bodies in what is called allostasis. Allostasis is the process by which our bodies actively maintain homeostasis despite everyday stressors (Sterling, 1988).

How Do We Measure Stress

There are two major approaches to measuring stress in humans. We can measure stress through self report measures, which ask individuals to rate the presence of affective and physiological symptoms that commonly occur in response to stress. We can also measure stress through biological markers which are chemicals in the body that change in concentration as a result of exposure to stress (as detailed in section “Defining Psychosocial Stress”).

Biological Measures: Cortisol

Cortisol as mentioned previously is a glucocorticoid in humans that plays a role in the chemical communication of the HPA axis. They are steroid hormones that regulate metabolic, cardiovascular, immune, and behavioral processes and are the end product of the HPA axis (Charmandari, et al., 2005). Thus, it has been posited that basal levels of cortisol serve as a valid indicator of change in stress level (Pruessner, et al., 1997). However many factors influence cortisol levels including: age, gender, oral contraceptives,

sleep patterns, caffeine, alcohol, and tobacco. Furthermore, dissociations between HPA axis activity and cortisol levels have been observed which further obscures the meaning of this widely used biomarker (Hellhammer, Wust, & Kudielka, 2009). Clearly, careful examination of these confounds must be completed in order to make valid interpretations of this biomarker. Nonetheless, cortisol can serve as an extremely useful objective marker of stress if all possible confounds are accounted for properly (Hellhammer, et al., 2009).

Biological Measures: Alpha Amylase

Alpha amylase is being studied as a potential biomarker of autonomic activity. The autonomic system is activated in response to stress and is the other major system involved in the response along with the HPA axis. It is generally believed that norepinephrine stimulates the sympathetic system which leads to higher levels of protein, specifically alpha amylase (Baum, 1993). Conversely, it is believed that higher rates of fluid output are the result of acetylcholine stimulation of the parasympathetic cholinergic system (Baum, 1993). However, both parasympathetic and sympathetic activation result in increased levels of alpha amylase (Nater, et al., 2005). Nater and colleagues (2005) note that increases in alpha amylase have been found in response to physical stressors such as: treadmill exercise, exposure to a high pressure chamber, running, bicycle exercise, or cold exposure (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; S. Gilman, Thornton, Miller, & Biersner, 1979; S. C. Gilman, Fischer, Biersner, Thornton, & Miller, 1979; Nexø, Hansen, & Konradsen, 1988; Steerenberg, et al., 1997; Walsh, et al., 1999). Alpha amylase levels increase in response to psychological stressors as well (Bosch, et al., 1996), although the response is less consistent than observed in response to physical stress (Nater, et al., 2005). However, many of the studies finding inconsistent results used atypical

methodological procedures, which makes valid comparison to previous studies difficult (Chatterton, et al., 1996). Studies using standardized laboratory settings and well researched psychological stressors such as the Trier Stress Test, have found consistent rises in levels of Alpha Amylase in response to acute stress (Nater, et al., 2005).

Subjective Measures of Stress

Subjective Measures: Perceived Stress Scale

The vast amount of scientific research on stress focuses on a very idiosyncratic or specific facet of stress such as a medical or mental health condition. Therefore, stress is often measured indirectly through depression, quality of life, and anxiety measures. Measures of these constructs often account for stress within their total scales. In looking at more specific subjective report stress measures one instrument dominates the field, the Perceived Stress Scale. The perceived Stress Scale was developed in 1983 to assess individual's perceptions of the stressful events in their lives over the past month (S. Cohen, Kamarck, & Mermelstein, 1983). The scale is available in three versions including 4, 10, and 14 item scales. The 10 item scale has shown the highest internal reliability with a Coefficient alpha of .78 (S. I. Cohen, 1988). The scale has 5 response options ranging from (never experiencing stress) as the result of a particular event in the past month to (very often experiencing stress). The perceived stress scale has been noted as the most widely used self report measure of stress in psychological research (Roberti, 2005).

Subjective Measures: A Daily Stress Inventory

The Daily Stress Inventory assesses stress in the past 24 hours through a checklist of stressful life events and situations. The original measure includes 60 items divided into

two subscales. One subscale measures interpersonal stress and one measures environmental hassles. This scale was designed to assess everyday stressful life events rather than less common traumatic life events. Participants score each item on a 7-point Likert scale ranging from 1 to 7, with anchors ranging from (event occurred but did not cause stress) to (event occurred and I panicked), if the event did not occur a score of 0 is given. The scale has an internal reliability of .83 (Brantley, Waggoner, Jones, & Rappaport, 1987). The Daily Stress Inventory has shown convergent validity with biological measures of stress (i.e. cortisol) (Brantley, Dietz, McKnight, Jones, & Tulley, 1988).

How Do We Scientifically Induce Stress

There are two major ways to induce stress in a laboratory setting. Stress can be induced through the completion of a psychological stressor such as public speaking task. Stress can also be induced through a physical activity such as treadmill exercise or exposure to pain. Commonly used tasks from each of these categories will be described in further detail.

Psychological Stressor: Trier Stress Test

The Trier stress test is a moderate psychological stress inducing task which includes a mental arithmetic task followed by a mach personal interview, both of which are completed in front of a panel of confederates who are believed to be judging the individuals performance. In six original independent studies performed by Kirschbaum and colleagues (1993) the task has been found to induce considerable changes in the concentration of cortisol serum in saliva as well as significant increases in heart rate. The

test has been found to reliably produce these hormonal and cardiovascular effects in 70 to 80% of participants (Kirschbaum, et al., 1993).

Physiological Stressor: Cold Presser Test

The cold pressor task is a physiological stressor which involves placing one's hand in cold water. This induces a slowly building pain of mild to moderate intensity and is alleviated upon voluntary withdrawal of the hand. The procedure activates thermal and nociceptor receptors and elicits a stress response (Lovallo, 1975; McRae, et al., 2006). However, it has been noted that the task also includes a social psychological component because the experimenter stays in the room to evaluate how long the limb is held under water by the subject (Schoofs, Wolf, & Smeets, 2009). This task has been shown to reliably increase activity of the sympathetic nervous system as well as the HPA axis (Lovallo, 1975).

Animal Models of Stress Induction

In addition to the widely utilized stress tests used in humans there are a number of tests used primarily in animal models. These tests include tail pinch, social defeat, neonatal isolation, food restriction, sleep deprivation, exogenous injections of stress hormones, and surgeries altering the physiological stress response.

The Role of Stress in Addiction

Stress in Addiction Acquisition

Animal studies have provided evidence that stress and the subsequent activation of the HPA axis has a strong effect on the acquisition of drug self administration and drug

seeking behavior (Goeders, 2003). Goeders and colleagues (2003) note that cocaine and amphetamine self administration in rats is increased by many regularly used laboratory stress paradigms including tail pinch (Piazza, Deminiere, le Moal, & Simon, 1990), social defeat (Tidey & Miczek, 1997), and neonatal isolation (Kosten, Miserendino, & Kehoe, 2000). Animals given exogenous corticosterone injections evinced a sensitized response to low levels of cocaine (Mantsch, Saphier, & Goeders, 1998). When surgery to inhibit the final step of HPA axis activity was performed on rats they completely stopped self administration of cocaine at all levels tested, yet they still learned to respond effectively to receive food pellets, indicating different reward mechanisms mediating these two behaviors (Goeders & Guerin, 1996). Similar results were found when rats were pretreated with the corticosterone synthesis inhibitor ketoconazole (Campbell & Carroll, 2001). It has been posited that this counter intuitive increase in drug self administration involving what is normally a negative stimulus (stress) is due to a sensitization process involving dopamine, similar to what has been observed in drug dose related sensitization (Goeders, Irby, Shuster, & Guerin, 1997) (Piazza and LeMoal, 1998).

Due to ethical concerns we cannot induce substance dependence in humans; therefore we must look at correlational data to gain insight about stress and its relation to the initiation of addiction. Stress has been associated with the increased use of many substances in humans including: alcohol (Tyssen, Vaglum, Aasland, Gronvold, & Ekeberg, 1998), cocaine (Brady, Dansky, Back, Foa, & Carroll, 2001; Dansky, Byrne, & Brady, 1999), opioids (Sinha, 2001), nicotine (Siqueira, Rolnitzky, & Rickert, 2001), and cannabis (Butters, 2002). Increased substance use and substance dependence have been associated with many known stress related conditions or stressful situations such as Post Traumatic

Stress Disorder (Volpicelli, Balaraman, Hahn, Wallace, & Bux, 1999), unhappy marriage (Jose, van Oers, van de Mheen, Garretsen, & Mackenbach, 2000), dissatisfied employment (Jose, et al., 2000), harassment (Richman, Flaherty, & Rospenda, 1996; Rospenda, Richman, Wislar, & Flaherty, 2000), and work stress (Vasse, Nijhuis, & Kok, 1998). Thus, the connection between stress and substance use disorders is particularly important given that it may play a role not only in the treatment and prevention of relapse but also in the acquisition of substance use disorders.

Stress in Addiction Maintenance

Pre clinical (G. F. Koob, et al., 2004) and clinical studies (Adinoff, et al., 2005a, 2005b; Kreek & Koob, 1998) have shown that acute withdrawal from addictive substances is associated with hyper activation of the HPA-axis and causes stress related feelings. This acute withdrawal can occur daily in a dependent individual between administrations of the addictive substance. The increased stress associated with acute withdrawal from a substance is negatively reinforcing and therefore motivational for the individual to maintain use of the substance.

Stress in Addiction Relapse

Following the acute withdrawal phase from a substance, exposure to stressors increases the likelihood of reinstatement of the substance (S. Cohen & Lichtenstein, 1990; Swan, et al., 1988). The neuronal pathways involved in the stress induced motivation to reinstate substance use due to acute withdrawal overlap with the neural circuitry activated by stressors during times of withdrawal free abstinence (Shaham, Shalev, Lu, De Wit, &

Stewart, 2003). Stress is often noted as the most common variable leading to relapse in humans (Bossert, Ghitza, Lu, Epstein, & Shaham, 2005).

The Role of Stress in Nicotine Dependence

Stress: Nicotine Acquisition

In considering how stress may play a role in the acquisition of nicotine dependence it can be helpful to consider the demographic data regarding the age that the majority of people start using nicotine. Specifically we know that many individuals who are smokers begin smoking in adolescence or in college (age 18-25) (Gilpin & Pierce, 1997). Among these groups there is evidence that stress often precipitates the onset of smoking initiation (Lamon & Alonzo, 1997). There are several theories as to why smoking acquisition and stress may be connected. While debated in the literature, there is some evidence to suggest that nicotine has anxiolytic properties (Tucci, et al., 2003). If this is the case, nicotine may play a role in reducing the experience of distress or stress and therefore increase the likelihood of use. This theory fits well with the basic principles of the self medication theory of addiction detailed in the previous section “Substance Use: Self Medication”.

Stress: Nicotine Maintenance

As with most addictive substances, acute withdrawal from nicotine initially increases activity in the HPA-axis (Richter & Weiss, 1999). This increase in HPA-axis activity is thought to influence motivation to reinstate the substance via negative reinforcement (G. F. Koob, 2009). This negative reinforcement occurs when the unpleasant feelings of withdrawal are ended by reinstatement of the addictive substance. As mentioned previously, some researchers suggest that this negative reinforcement causes the

instatement of nicotine to be anxiolytic in nature. Increased stress has also been shown to be positively correlated with urges to smoke (Swan, Ward, Jack, et al., 1993; Tiffany, 1990). Laboratory experiments have shown that CRF antagonist injections block the anxiogenic like effects from withdrawal from nicotine, indicating that it is this stress system that is responsible for much of the anxiety and stress related to withdrawal (Tucci, et al., 2003). Motivation to maintain smoking behavior may also be reinforced by increased sensitivity to drug exposure during times of stress (Goeders, et al., 1997). The increased sensitivity to lower levels of nicotine in response to stressors was blocked by CRF antagonists (Bruijnzeel, Zislis, Wilson, & Gold, 2007), lending further support for the role of the HPA-axis in maintaining smoking behaviors.

Stress: Nicotine Relapse

Hyper activation of the HPA-axis in response to stressors has been noted in formerly nicotine dependent individuals, even after the acute withdrawal period (Tucci, et al., 2003). This increased reactivity to stress may play a pivotal role in causing relapse in individuals who have already made it past the acute withdrawal phase. People often report that exposure to stressful life events directly lead to their smoking relapse (Baer & Lichtenstein, 1988; Cummings, Jaen, & Giovino, 1985; Shiffman, 1982).

Psychophysiology

The underlying assumption/principle of psychophysiology is that there is a corresponding physical event or cascade of events that occur for every behavior, whether it is an action, cognition, or emotion. These events involve the chemical and electrical properties of the nervous system, and can be measured through a number of techniques.

Galvanic Skin Response

Change in galvanic skin conductance is controlled by the sympathetic nervous system and is associated with physiological arousal (Duffy, 1957). Galvanic skin conductance increases when a participant processes any novel stimuli, this is posited to be an orienting response (M. M. Bradley, 2009). These changes are even larger when participants process novel pleasant or novel unpleasant stimuli as compared to novel neutral stimuli (M. M. Bradley, 2009). If presented with the same stimuli a week later, skin conductance increases again for unpleasant and pleasant stimuli compared to neutral pictures (M. M. Bradley, et al., 1993). The repetition of neutral stimuli abolishes significant changes in skin conductance implicating novelty as the mediator of the response (M. M. Bradley, 2009). When unpleasant or pleasant stimuli are processed repeatedly within the same experimental session changes in skin conductance will attenuate, though not as quickly as with neutral stimuli (M. M. Bradley, et al., 1993).

Electrocardiogram Response

Early studies demonstrated that exposure to novel stimuli results in a prolonged decrease in heart rate (Graham & Clifton, 1966; Lacey, 1967). Bradley and colleagues (2009) determined that novel pictures elicit a deceleration in cardiac activity during the first 2 seconds of stimulus presentation for pleasant unpleasant, and neutral, images. The researchers also determined that unpleasant stimuli evoked a significantly larger decrease in heart rate compared to pleasant or neutral images. This deceleration indicates increased perceptual intake, an intense focus, for these unpleasant cues. Like the results from galvanic skin response studies, readministration of the stimuli one week later resulted in no significant changes in heart rate across all picture types including no decreased activity to

unpleasant stimuli as compared to pleasant and neutral stimuli (M. M. Bradley, et al., 1993). It was also noted that when stimuli are presented repeatedly within the same experimental session, changes in heart rate depending on stimulus type become insignificant.

Affective Modulation of the Startle Response Utilizing: Orbicularis Oculi Muscle

In most animals a sudden intense stimulus will evoke a startle response, the involuntary contraction of a series of flexion and most skeletal muscles throughout the body (Landis, 1939). This muscle contraction serves a defensive purpose, allowing for an animal to escape or reduce physical harm in the event of a threat. Gross features of the startle response include a forward thrusting of the head and a descending flexor wave reaction extending through the trunk to the knees (Lang, Bradley, & Cuthbert, 1990). The primary and most stable event in this cascade of action is the sudden closing of the eyelids, an eye blink, which Landis and Hunt originally elucidated in humans by firing a pistol. The eye blink is often accompanied by contraction of the orbicularis oculi muscle. This reflexive contraction occurs 30 – 50 milliseconds after the onset of a sudden acoustic stimulus (Lang, et al., 1990). This reflex habituates normally with short interstimulus intervals; however it dishabituates quickly and therefore can be stimulated repeatedly during a brief period (Lang, et al., 1990). It is common to evoke as many as 50 reflexes in a 30 minute trial (Geier, et al., 2000). This response has been recorded in a number of ways including photography, a potentiometer attached with a thread to the eyelid, by the electro-oculogram with abrupt pen movements indicating the lid passing rapidly over the corneal surface, or by electromyographic measurement of the orbicularis muscle. Electromyographic measurement is used by most researchers because “it captures events

most proximal to the neural path of innervations” (Lang, et al., 1990). The startle response has been used by researchers to examine emotional responses to novel stimuli. This approach categorizes behavior as appetitive/pleasant, neutral, or aversive/unpleasant. When the startle response is elicited while an individual is viewing an experimental cue that is inherently pleasant to them their reflexive response is diminished. Conversely, when the startle response is elicited while an individual is viewing an image that is inherently unpleasant or aversive to them the reflexive response is increased. That is, appetitive stimuli decrease the startle response while aversive stimuli increase the response. The potentiation of the startle response while viewing aversive stimuli and attenuation while viewing appetitive stimuli has been replicated repeatedly (M. M. Bradley, 2009; Dempsey, Cohen, Hobson, & Randall, 2007; Geier, et al., 2000; Hamm, Cuthbert, Globisch, & Vaitl, 1997; Lang, et al., 1990).

Affective Modulation Neuropathway

The neural circuitry underlying modulation of the startle response during appetitive and aversive processing has been systematically investigated and a basic requisite pathway has been elucidated. The startle stimulus activates the cochlear nucleus and proceeds to the pontine reticular formation; the impulse is then carried through spinal neurons to the reflex effectors (M. L. Bradley, P., 2007) . There is a secondary circuit intersecting the primary reflex pathway which increases the amplitude of the orbicularis reflex after fear conditioning (M. L. Bradley, P., 2007). Strong evidence suggests that the amygdala is responsible for mediating this effect through its direct projections to the nucleus reticularis pontis caudalis. This has been evinced through electrical stimulation of the amygdala’s central nucleus which increases the amplitude of the startle response, through Positron

Emission Tomography studies, and through lesion studies of the amygdala which eliminate startle potentiation (Davis, 1989). While there is large individual variability in overall startle magnitude at baseline, this does not affect the affective or emotionally based modulation of the startle magnitude (M. M. Bradley, et al., 1993).

Affective Modulation of the Startle Response Utilizing: Post-Auricular Muscle

The postauricular reflex has recently been introduced as a measure of appetitive responding to stimuli (Benning, Patrick, & Lang, 2004). This response is produced by the vestigial muscle, and retracts the ear dorsally during exposure to a loud abrupt acoustic stimulus. Initial studies of the postauricular reflex established that the amplitude of the response was significantly increased during processing of pleasant stimuli in comparison to neutral and unpleasant stimuli (Benning, et al., 2004), and these results have been replicated (Hess, Sabourin, & Kleck, 2007). The postauricular response can be measured simultaneously with the orbicularis oculi, meaning they can both be elicited independently by the same acoustic stimulus. The postauricular reflex has been shown to have a faster reaction time to acoustic stimuli than the orbicularis (9 – 11ms versus 30 – 50ms) (Hackley, 1993; Lang, et al., 1990). While research utilizing this physiological response as an appetitive index is in its infancy, the current findings are promising.

Affective Modulation of the Startle Response in Nicotine Dependence

The affective modulation of the startle response has been proposed as an objective measure of motivational valence for pictures of smoking cues (Geier, et al., 2000). The startle reflex has been used to examine the effects of nicotine on the physiological responsiveness of the muscular system for some time, e.g. (Gilbert & Hagen, 1980). However, the startle response was not used to examine the emotionally based aversive or

appetitive nature of smoking pictures until Geier and colleagues do so in 2000. There has been much debate as to whether smoking cues are aversive in nature, negatively reinforcing through increase in withdrawal state; or conversely appetitive in nature, positively reinforcing through pleasurable effects of the drug. Self report measures of smoking cues indicate that they increase desire to smoke a cigarette and do so without being described as unpleasant (Mucha, Geier, & Pauli, 1999). This finding supported the appetitive nature of smoking cues found previously by a number of researchers (Lazev, Herzog, & Brandon, 1999; Mucha, Geier, Stuhlinger, & Mundle, 2000). These findings are incongruent with the conventional pharmacological and evolutionary models of drug cues which postulate that cues provoke aversive reactions through a subjective increase in withdrawal like state (G. F. Koob & Nestler, 1997; Siegel, 1975).

Geier and colleagues (2000) found that smoking cues attenuated the startle response in dependent smokers as compared to neutral and unpleasant pictures, indicating that the cues were appetitive in nature. A pseudo replication study conducted shortly thereafter, (Orain-Pelissolo, Grillon, Perez-Diaz, & Jouvent, 2004), failed to replicate the attenuated startle response during exposure to smoking cues. It is imperative to note that there were several important differences in methodology between these studies. The Geier (2000) study included participants diagnostically determined to be nicotine dependent and used standardized images from the International Affective Picture System. Conversely, the Orain-Pelissolo (2004) study used moderately nicotine dependent individuals and unstandardized images obtained through searches conducted on the World Wide Web. The findings of the Geier 2000 study were replicated by Dempsey and colleagues (2007),

supporting the previous research that smoking cues attenuate the startle response in nicotine dependent individuals.

Careful attention to specific methodology is critical in ensuring valid results in the affective modulation of the startle response paradigm. Failure to follow specific methodology has resulted in the inconsistent results mentioned earlier. One of the most important methodological points that must be followed in order to obtain accurate results is the use of standardized images. The International Affective Picture System is a database of normed emotional stimuli developed for experimental investigations of emotion and attention, and is recommended by the Society for Psychophysiological Research.

While we have an excellent database of standardized emotional images we are currently lacking a standardized database of smoking and drug related cues. This is particularly important given that there is a wealth of research suggesting that the specific characteristics of cues may be influential in their appetitiveness to participants. For example, recent research regarding nicotine related visual stimuli found that characteristics such as the length of the cigarette smoked in the cue elicited significantly different responses in participants (Mucha, et al., 2000). The influence of similar specific facets of nicotine cues such as cigarette brand, model orientation (1st person vs. 3rd person viewpoint), and gender of the model smoking are currently being studied.

CHAPTER III

METHODOLOGY

Participants

Twenty-nine nicotine dependent individuals (15 in stress condition, 14 controls) participated in the current study. Current smoking status was biologically confirmed with an average carbon monoxide (CO) expired breath reading of 13.70 ppm. Most dependent smokers emit levels of 10ppm or more (Cox & Whichelow, 1985). A self reported average of 3.1 on the Fagerstrom Test for Nicotine Dependence was observed indicating moderate to high levels of nicotine dependence (Heatherton, 1991). Stress and control groups did not differ in terms of CO reading, Fagestrom Test for Nicotine Dependence score, number of cigarettes smoked daily, amount of time since last cigarette, ethnicity, age, gender, or education. Exclusion criteria included dependence on any other drug than nicotine or caffeine, previous diagnosis of a psychotic disorder, reported hearing or visual difficulties, and positive alcohol breathalyzer reading. No participants were excluded due to these conditions.

Measures

Trier Social Stress Test (Kirschbaum, et al., 1993)

The Trier Social Stress Test is a psychological stress inducing task which includes mental arithmetic followed by a mock personal interview, both of which are completed in front of

a panel of confederates who are believed to be judging the individual's performance. In six original independent studies performed by Kirschbaum and colleagues (1993) the task was found to induce considerable changes in cortisol (an indicator of HPA-axis activity) as well as significant increases in heart rate and other measures of sympathetic nervous system activity (Kirschbaum, et al., 1993). The control condition will include viewing of a physiologically neutral video.

Fagerstrom Test for Nicotine Dependence (Heatherton, Kozlowski, Frecker, & Fagerstrom, 1991)

The Fagerstrom Test for Nicotine Dependence (FTND) is a 6-item self-report measure of nicotine dependence. Scores can range from 0 to 10, with increasing values suggesting higher levels of nicotine dependence. The total score on the FTND has been shown to have adequate reliability (Cronbach's $\alpha = .64$) and is correlated with biological markers of nicotine (C. S. Pomerleau, et al., 1994). Scores of 3-4 on the Fagerstrom Test for Nicotine Dependence indicate low-moderate dependence to nicotine (Heatherton, 1991).

Carbon Monoxide Level (CO)

Smoked tobacco causes a buildup of the poisonous chemical carbon monoxide in the lungs. Measuring the amount of carbon monoxide in the air expired by an individual provides a short term estimate of smoking (Leone, 2005). Dependent nicotine users typically have Carbon Monoxide Levels above 10 parts per million (ppm) (Cox & Wichelow, 1985). The expired breath of participants was measured using a Bedfont Scientific Limited Compact Smokerlyzer Model A100c.

The Perceived Stress Scale 10 Item (S. Cohen, et al., 1983)

The Perceived Stress Scale (PSS) is a 10 item self report scale of symptoms of stress. The scale uses a 5 point Likert scale to assess the frequency of specific symptoms of stress with anchors ranging from “never” to “very often”. The scale has adequate internal reliability with a reported Crohnbach’s alpha of .78 (S. I. Cohen, 1988). The current study used a modified form of the PSS in which the time frame was changed to reflect symptoms of stress within the past hour. The scale was given at baseline and again after the TSST. Crohnbach’s Alpha for the scale was .89 at baseline and .84 after the TSST, indicating good internal reliability for the modified scale.

Physiological Data Acquisition

Electromyography

Ag – Agl 4 mm surface electromyography electrodes (In Vivo Metric, E220 – LS), were attached to the skin above the orbicularis oculi of the left eye in a bipolar configuration, and a ground reference electrode was attached to the medial phalanges of the left hand. Skin above the orbicularis oculi was abraded with Nuprep abrasive skin prepping gel and electrodes were filled with Signa electrode gel. An amplification setting of 50,000 on a Biopac MP150 bioamplifier was used to collect raw electromyography data. Rectification of the raw signal using a bandpass filter setting of 8 – 150hz, and a time constant of 10ms, was completed on a MP150 EMG 100c integrator. The rectified signal was then manually scored using Acqknowledge scoring software. Startle scores were calculated by subtracting the baseline eye blink magnitude (data point just before startle reflex) from the peak electromyographic magnitude between 21 and 200ms after the

acoustic probe was administered. Consistent with previous procedures published in affective modulation of the startle response to nicotine cues literature (Dempsey, et al., 2007) trial rejection included (1) excessive noise (>19 uV) during baseline or (2) non-startle blink activity occurring at zero to 21ms after probe administration. Participants were excluded if they failed to show a reliable startle response (no regular increase in startle magnitude 21 - 200ms after probe onset). Five participants were removed from the electromyographic analysis due to an unreliable startle response. Impedance was measured with Checktrode model 1089, and was required to be below 10ku for all physiological measurements.

Skin Conductance

Biopac EL507 skin conductance electrodes were placed on the medial phalanges of the second and fourth digits of the left hand. Skin was prepped with distilled water and electrodes were pre-filled by the manufacturer with isotonic gel. A transduction range of 0-20 μ S on a Biopac MP150 GSR100C skin conductance amplifier was used to collect tonic skin conductance data. The resulting skin conductance signals were then manually scored using Acqknowledge scoring software. Mean skin conductance level in microsiemens was measured for the 12 minutes immediately following the experimental manipulation and averaged in three segments of four minutes each. Phasic skin conductance values were derived from the tonic signal and then obtained by calculating the change in skin conductance that occurred between one and four seconds after stimulus onset. This time period has been shown to indicate stimulus evoked skin conductance response (Andreassi, 2000).

Heart Rate

Biopac EL503 EKG electrodes were placed on the left and right forearms. Skin was prepped with alcohol, and electrodes were filled with Signa electrode gel. An amplification setting of 1000 on a Biopac MP150 bioamplifier was used to collect heart rate data. Event related heart rate was calculated by subtracting the mean heart rate in a one second window immediately preceding stimulus onset from the mean heart rate in a four second window between two and six seconds after stimulus onset. Orienting effect was calculated by subtracting the mean heart rate in a one second window immediately preceding stimulus onset from the mean heart rate in a two second window immediately following stimulus onset.

Procedure and Stimulus Materials

Directly following either the stress (Trier Social Stress Test) or control condition (physiologically neutral video), participants were seated in a chair approximately 24 inches from a 17 inch Dell computer monitor. Participants viewed 12 pleasant, 12 unpleasant, and 12 neutral images standardized by the International Affective Picture System (IAPS), as well as 12 nicotine cue images. The pleasant pictures are composed of IAPS numbers 4141, 4180, 4232, 4310, 4311, 4559, 4658, 4659, 4664, 4670, 4680, and 4683. Some of these images are erotic in nature, which has been shown to produce the greatest inhibition of startle (Bernat, Patrick, Benning, & Tellegen, 2006). The unpleasant pictures include IAPS image numbers 1050, 1120, 1300, 1321, 1525, 1931, 6230, 6243, 6244, 6250, 6300, and 6510. These are images of humans and animals in threatening postures, with threat pictures being shown to produce the greatest startle potentiation (M. Bradley, Cuthbert, B.,

& Lang, P., 1999). The neutral pictures include IAPS image numbers 7000, 7010, 7020, 7030, 7041, 7050, 7052, 7055, 7175, 7179, 7217, and 7235. The neutral images include common household objects (e.g., spoon, basket, hairdryer). Twelve nicotine cues were also presented. The nicotine cues were created to control for brand, phase of consumption, gender of smoker, and orientation (Geier, et al., 2000).

An acoustic startle probe (50 ms in duration, instantaneous rise-time [$<1\text{ms}$]) was binaurally administered during ten out of the twelve trials in each category, resulting in contraction of the orbicularis oculi. A probe latency of approximately four seconds was chosen as previous research has documented that at a latency of three seconds, affective modulation magnitude reaches asymptote (M. M. Bradley, et al., 1993). To control for effects of participant's expectancy in startle probe timing, a four second onset was used as an average (range of presentation three to five seconds). In order to ensure participant safety as well as consistency in probe intensity, sound level was verified at 105dB prior to each experimental session, via a Digital Sound Level Meter. The order of picture category-type was randomized to ensure equal distribution across five different orders. Measures of skin conductance and cardiac activity were taken concurrently with measures of electromyography. Participants were instructed to watch the image the entire time it was displayed. Participants completed a five-minute adaptation period in which they sat motionless, watching a blank screen while 10 startle probes were administered. Participants were then shown the series of 48 pictures (detailed previously) for six seconds each, with a variable 15-20 second inter-trial interval.

Data Analysis

To examine whether there were group differences in SNS responding, a 2 (group: stress, control) x 3 (time: first four minute segment, second four minute segment, third four minute segment) repeated measures ANOVA was conducted on tonic skin conductance levels. Electromyographic data were standardized within subject and then averaged by stimulus category, consistent with previous research in the field (Blumenthal, 1998; Dempsey, et al., 2007). A series of 2 (group: stress, control) x 4 (stimulus category: positive, neutral, negative, smoking) repeated measures ANOVAs were conducted on orbicularis oculi electromyographic magnitude, phasic skin conductance average, and heart rate beat per minute difference scores. In line with previous research in the field with similar design (Dempsey, et al., 2007), a modified Bonferroni correction was used to control for alpha in all post hoc analyses (Keppel, 1991).

CHAPTER IV

FINDINGS

The perceived stress scale scores increased significantly from baseline to time two in the stress group, $F(1, 28) = 4.531, p = .042$, but not in the control group, $F(1, 24) = .007, p = .935$. This indicates that the stress condition significantly increased participants self reported level of stress whereas the control condition did not.

A significant decrease in skin conductance level over time was observed, $F(2, 26) = 9.886, p = .001$. Skin conductance was significantly lower in the third time interval than in the first (mean difference = $.769, p = .001$) and second (mean difference = $.528, p < .001$) time intervals. However, the group by time interaction was not significant, $F(2, 26) = .123, p = .885$. No group differences were observed in overall skin conductance level, $F(1, 27) = .250, p = .621$.

Startle reflex analysis revealed that cue type was significant, $F(3, 20) = 10.502, p < .001$. Startle magnitude was significantly larger while viewing unpleasant images than while viewing pleasant, $F(1, 18) = 42.153, p < .001$, smoking, $F(1, 18) = 51.406, p < .001$, and neutral images, $F(1,18) = 19.021, p < .001$ (Figure 1). The stress group evinced a diminished startle response mean ($-.294$) as compared to the control group ($-.246$) for smoking images, however the cue type by group interaction was not significant, $F(3, 20) = .291, p = .832$ (Figure 2). A One Way ANOVA was conducted on group startle data to smoking images, in order to examine the effect of smoking cues between groups without

losing power in the analysis of the other stimuli. No significant effect was observed for smoking related stimuli between groups in this analysis $F(1, 24) = .891, p = .380$. No significant differences in overall startle were observed between groups, $F(1, 22) = .006, p = .937$. Means and standard deviations of EMG by cue and group are listed in Table 1.

For the phasic skin conductance data, cue type approached but did not reach statistical significance, $F(3, 22) = 2.549, p = .082$. Group by cue type interaction was not significant, $F(3, 22) = .725, p = .543$. Group differences in overall skin conductance response approached but did not reach significance, $F(1, 24) = 3.759, p = .064$.

Cue related heart rate data analysis, which assessed heart rate responding between two and six seconds after cue onset, revealed no significant effect of cue type, $F(3, 22) = .035, p = .991$. Cue by group interaction was also not significant, $F(3, 22) = .411, p = .747$. The orienting response, which assessed heart rate responding during the first two seconds of cue onset, was not significant, $F(3, 22) = .381, p = .768$, cue by interaction was also not significant, $F(3, 22) = 1.609, p = .216$. No group differences were observed overall in either heart rate analysis, $F(1, 24) = .012, p = .913$; $F(1, 24) = .223, p = .641$.

CHAPTER V

CONCLUSION

The current study assessed the modulation of the startle reflex, heart rate, and skin conductance to smoking cues as a function of stress amongst nicotine dependent individuals. Previous research indicating that nicotine cues are reliably appetitive in nature promotes the affective modulation of the startle response paradigm as a potentially valuable tool for elucidating mechanisms involved in nicotine dependence. Results of the current study replicate past research indicating that smoking cues diminish the startle response in nicotine dependent individuals (Dempsey, et al., 2007; Geier, et al., 2000). The present study extended upon previous research by examining the effects of an experimental manipulation on the physiological responding of participants.

Specifically, participants were randomly assigned to a stress (Trier Social Stress Test) or control (physiologically neutral) condition. Stress was chosen as a variable of interest due to the plethora of previous research indicating a relationship between this negative affective state and nicotine dependence (Bruijnzeel, et al., 2007; Goeders, 2003; G. F. Koob, 2010; Richter & Weiss, 1999; Swan, Ward, Carmelli, & Jack, 1993). Results revealed that the cue by group interaction was not significant, suggesting that the experimental manipulation did not influence the magnitude of the startle response.

However, means were observed in the direction consistent with the hypothesis that participants in the stress condition would evince a diminished startle response as compared to participants in the control condition. An examination of the startle response as a function of cortisol level may reveal more conclusive results. Sympathetic Nervous System Activity and HPA-axis activity are related, but can exert independent effects. The Trier Social Stress Test is designed to activate the HPA-axis specifically, not necessarily the Sympathetic Nervous System.

In order to determine if the stress condition was effective, we tested the hypothesis that participants in the stress group would report a significant increase in stress following the experimental manipulation whereas participants in the control group would report no significant changes in stress. This hypothesis was supported by participant responding on the Perceived Stress Scale. This analysis was followed by an examination of physiological changes that could be attributed to the stress condition.

We hypothesized that a significant decrease in skin conductance level over time would be observed in the stress group but not in the control group. This hypothesis was formed to examine any residual effects of the stress task on sympathetic nervous system activity. However, contrary to this hypothesis a significant decrease in skin conductance was observed over time for the entire sample. This may have been due to an increase in skin conductance related to experimenter proximity to the participant while connecting electrodes directly before data recording. The significant decrease was not likely due to sympathetic activation from the stress task as no group interaction was observed. However, these results do not indicate that the stress task failed to increase sympathetic activation during the actual administration of the task. The design necessary for testing the primary

hypothesis (modulation of the startle reflex) did not allow for the optimal design for testing changes in tonic skin conductance levels. This resulted in recording of the residual effects of the experimental manipulation rather than recording the effects during the manipulation. Thus, the design of the experiment may have led to the equivocal results observed.

We hypothesized that large phasic skin conductance responses would be observed when participants viewed smoking images, and that the largest skin conductance responses to smoking images would be observed in the stress group. This hypothesis was based on previous research which demonstrated that participants evince significantly larger skin conductance responses to both pleasant and unpleasant images as compared to neutral images, indicating increased physiological arousal to these images (M. M. Bradley, 2009). Images of nicotine should be viewed as pleasant to nicotine dependent individuals based on previous research in the affective modulation of the startle reflex field (Geier 2000, Dempsey 2009). Differences in skin conductance response to cue type approached but did not reach significance. In line with previous research, the largest increases in skin conductance were observed while participants viewed pleasant and negative images (M. M. Bradley, 2009). Increases in skin conductance to smoking images were equivalent to the small increases observed while participants viewed neutral images, contrary to the hypothesis that smoking cues would evoke the largest increases in skin conductance. This indicates that smoking images did not produce phasic increases in sympathetic nervous system activated skin conductance. However, previous literature has shown that novel images of any type produce elevation in skin conductance (M. M. Bradley, 2009). Images in the smoking category were relatively similar to each other as compared to images in the other three categories. Therefore, one possibility for the results observed is that images

within the smoking group lost their novelty after the first few presentations. The other categories of images were likely dissimilar enough to maintain a high degree of novelty, resulting in a comparatively blunted physiological arousal for the smoking group.

It was hypothesized that a significant increase in heart rate would be observed when participants viewed smoking images as previous research has shown this effect in pleasant images (M. M. Bradley, 2009). Heart rate change to image category was not significant for the sample as a whole or within either condition. This analysis was repeated with heart rate standardized within participant, however no significant findings were observed. These results suggest that neither positive and negative images, nor smoking images modulated heart rate as previous researchers have reported (M. M. Bradley, 2009). The failure of the current study to find results similar to those reported by previous researchers may be due to differences in samples between these previous studies and the current study. Previous researchers have used nicotine free samples while the current study used a nicotine dependent sample. Nicotine has been shown to produce changes in measures of heart rate (Hayano, et al., 1990), which may account for the lack of replication observed in the current study.

The current study suggests that exposure to stress does not significantly modulate physiological responding to smoking cues. Therefore, smoking cues may not play a key role in the relationship between smoking and stress. While subtle differences in physiological responding to smoking cues were observed between groups, further examination is needed to understand the clinical significance of these differences. Designs including direct measurement of cortisol levels and their relation to the modulation of the startle reflex to nicotine cues will improve upon the current study. Given the insignificant

findings in the current study an alternative explanation for the behavioral mechanisms underlying the relationship between stress and smoking may involve interoceptive cues associated with activation of the HPA-axis. Interoceptive cues include the physical symptoms associated with HPA-axis activity and may be a more direct mechanism for priming nicotine administration than stimuli outside of the individual such as smoking cues. Furthermore, nicotine itself activates HPA-axis activity, so Sympathetic Nervous System and Peripheral Nervous System effects may have been the result of those systems compensating for the HPA-axis hyperactivity.

Limitations

This study included limitations that are important to consider when interpreting the results. HPA-axis activity could not be directly measured as cortisol analysis was not completed. Therefore results are dependent on the ability of the Trier Social Stress Test to reliably increase HPA-axis activation and the significant increase observed on the Perceived Stress Scale in the stress group to reflect HPA-axis activation. All physiological measures were recorded directly after the experimental manipulation; therefore only residual effects of the manipulation were examined. Examining the residual effects of the manipulation is an ideal method for analysis of the affective modulation of the startle response paradigm but may not be ideal for other measures of physiological responding. Finally, participants included college students enrolled in introductory psychology courses which may limit the ability for results to be generalized to other populations.

Future Directions

Overall, the affective modulation of the startle response paradigm may not have adequate specificity to determine fine differences in participant's responses to images. It may be unlikely that significant differences between varying degrees of pleasantness would be observed. That is, the affective modulation of the startle response is likely to discriminate pleasant images from unpleasant and neutral images but may be limited in its ability to adequately differentiate between responses to images within the same general category. Future studies including this paradigm should test the clinical significance of small changes in the startle reflex such as those observed in the current study. Future research should also examine the unity observed between different indices of physiological responding to smoking images. Specifically, it would be expected that smoking images would influence physiological responding across different modalities in the same way that pleasant images do. Finally, the ability of the affective modulation of the startle response to serve as a physiological measure of individual success in smoking cessation treatment should be analyzed as it could greatly improve objective measurement of treatment efficacy.

Table 1: Electromyographic Startle Response Data

Cue Type	Stress Group <i>M (SD)</i>	Control Group <i>M (SD)</i>	Total <i>M (SD)</i>
Pleasant	-.2411 (.1288)	-.2172 (.4461)	-.2292 (.3213)
Smoking	-.29445 (.1353)	-.24658 (.1809)	-.2705 (.1581)
Neutral	-.1804 (.2962)	-.2208 (.1573)	-.2006 (.2328)
Unpleasant	.0960 (.3099)	.0830 (.3163)	.0895 (.3063)

Figure 1: Cue Means; Stress and Control Groups

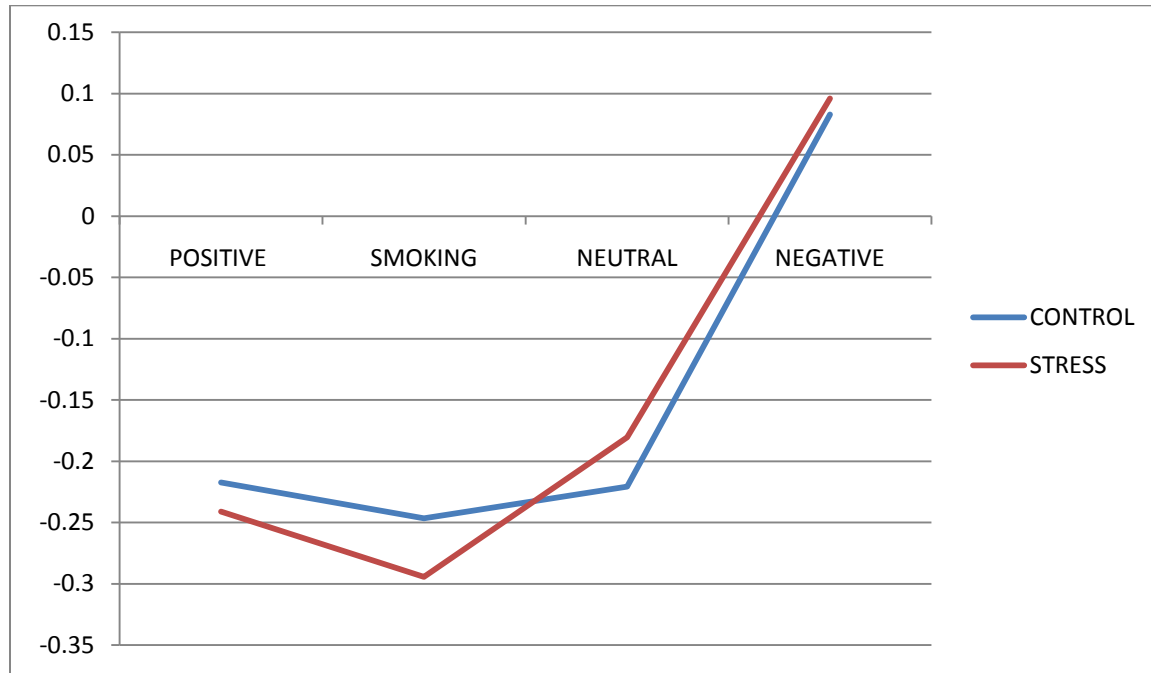
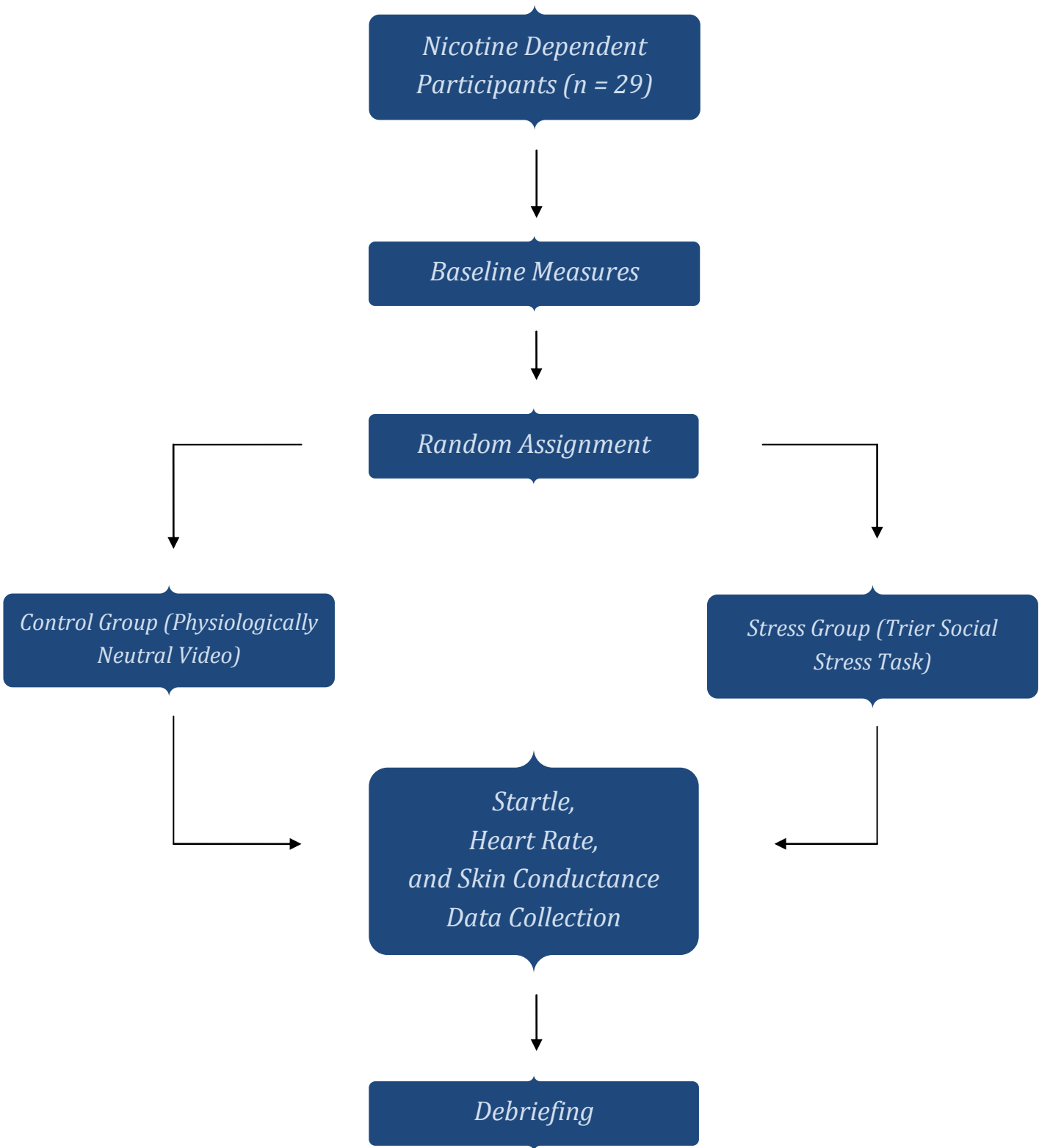


Figure 2: Experimental Design



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affect smoking motives and abstinence self-confidence among young adult smokers. *Addict Behav*, 31(3), 429-439.

APPENDICES

VITA

William V. Lechner

Candidate for the Degree of

Master of Science/Arts

Thesis: The Influence Of Stress On The Appetitive Nature Of Drug Cues, Skin
Conductance, And Heart Rate In A Nicotine Dependent Sample.

Major Field: Clinical Psychology

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Completed the requirements for the Master of Science in Clinical Psychology at
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Schuler, M., Lechner, W., Malcolm, R.J, Carter, R. (2009) Temporal and
demographic trends in concordance of urine drug Screens and self-reported use
in cocaine treatment studies. *Journal of Addiction Medicine*. 3 (4), 211-217.

Dempsey, J. P., Cohen, L. M., Watson, N. L., Lechner, W. V., Hobson, V.
L. & Smith, K. C. (2011). Blood pressure associated with the risk of alcohol
use disorder among smokers: Implications for treatment and screening.
Alcoholism Treatment Quarterly, 29: 123 – 131.

Name: William V. Lechner

Date of Degree: December, 2011

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Title of Study: THE INFLUENCE OF STRESS ON THE APPETITIVE NATURE OF
DRUG CUES, SKIN CONDUCTANCE, AND HEART RATE IN A NICOTINE
DEPENDENT SAMPLE.

Pages in Study: 71

Candidate for the Degree of Master of Science

Major Field: Clinical Psychology

Scope and Method of Study: The current study examined the influence of stress, a negative affective state intimately linked with nicotine use, on the affective modulation of the startle response to smoking cues.

Findings and Conclusions: Twenty-nine nicotine dependent participants were randomly assigned to a stress or control condition directly before administration of the affective modulation of the startle response paradigm. Both groups evinced significantly diminished startle magnitudes in response to nicotine cues as compared to threat images. A pattern indicating a greater decrease in startle magnitude means while viewing nicotine images for individuals in the stress condition was observed, however the cue by group interaction was not significant.

ADVISER'S APPROVAL: DeMond M. Grant
